

Inventors: Lockridge and Watkins
Serial No.: 09/748,739
Filed: December 26, 2000
Page 2

At pages 43 and 44, please amend the paragraph spanning pages 43 and 44 to read as follows:

02 One approach for targeting variant or heterologous nucleic acids to a single site in the genome uses Cre recombinase to target insertion of exogenous DNA into the eukaryotic genome at a site containing a site specific recombination sequence (Sauer and Henderson, Proc. Natl. Acad. Sci. USA, 85:5166-5170 (1988); Fukushige and Sauer, Proc. Natl. Acad. Sci. U.S.A. 89:7905-7909 (1992); Bethke and Sauer, Nuc. Acids Res., 25:2828-2834 (1997)). In addition to Cre recombinase, Flp recombinase can also be used to target insertion of exogenous DNA into a particular site in the genome (Dymecki, Proc. Natl. Acad. Sci. U.S.A. 93:6191-6196 (1996)). The target site for Flp recombinase consists of 13 base-pair repeats separated by an 8 base-pair spacer: 5'-GAAGTTCCTATTC[TCTAGAAA]GTATAGGAACTTC-3' (SEQ ID NO: 24). As described herein, the butyrylcholinesterases designated SEQ ID NOS: 4, 6, and 8, were obtained by transfection of variant libraries corresponding to region 5 of human butyrylcholinesterase (see, Table 2) into mammalian cells using Flp recombinase and the human 293T cell line. It is understood that any combination of site-specific recombinase and corresponding recombination site can be used in methods of the invention to target a nucleic acid to a particular site in the genome.

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At pages 58 and 59, please amend the paragraph spanning pages 58 and 59 to read as follows:

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Thirty-four variants were prepared using PCR-site directed mutagenesis of human butyrylcholinesterase DNA performed utilizing Pfu polymerase (Stratagene, La Jolla, CA). Three oligonucleotide primers were used to perform the mutagenesis. The mutagenesis primers were used at the same time as a general primer such as the SP6 promoter sequencing primer (MBI Fermentas, Amherst, NY) to amplify one end of the butyrylcholinesterase cDNA. The following primers were used to prepare the A328W mutant: A328W antisense 5' ATAGACTAAAAACCATGTCCCTTCATC 3' (SEQ ID NO: 29); T7 old sense 5' TAATACGACTCACTATAGGG 3' (SEQ ID NO: 30); and SP6 antisense 5' ATTTAGGTGACACTATAG 3' (SEQ ID NO: 31). The A328W primer spans 27 nucleotides and contains the A328W mutation in the middle of the primer. The PCR reaction products (megaprimers) were cleaned on QuiaQuick PCR (Qiagen, Santa Clarita, CA) according to the manufacturer's protocol to remove excess primers. The cleaned megaprimers were extended in a second PCR reaction to generate the complete 1.8 kb coding sequence of each of the 34 variants.

At page 77, please amend the first paragraph to read as follows:

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As shown in Table 5, several cell lines as well as other transfection methods were also characterized. As disclosed herein, Flp recombinase also can be used to target insertion of exogenous DNA into a particular site in the genome as described by Dymecki, supra, 1996. The target site for Flp recombinase